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An index technique to monitor broadcast calibration and bait pick up, plus rodent and avian sign under arid conditions†

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Abstract: As part of product-performance and wildlife-hazards studies of 2% zinc phosphide (Zn_3P_2) steam-rolled-oat baits (11.2 kg ha^{-1}) to reduce vole populations (*Microtus spp*) in alfalfa (*Medicago sativa*), we used randomly located, brushed-dirt plots (eight ~930–cm^2 plots per 0.2-ha enclosure) to monitor bait-broadcast and -removal patterns, as well as to index vole and avian sign. Research was conducted in 18 × 0.2-ha enclosures containing 2.5-year-old stands of alfalfa; a 2-day pre-bait (placebo baits broadcast in all enclosures) period followed by a 14-day test-bait period (placebo and 2% Zn_3P_2 baits in nine enclosures each) characterized the bait exposures. Baits were broadcast manually by two certified pesticide applicators (CPAs) using Spyker® Model-75 spreaders. Baits that fell onto plots were counted <30 min later to assess the uniformity of bait distribution. The main statistical design was a 2 (placebo or Zn_3P_2 baits) × 3 (vole-only, vole–pheasant, vole–quail exposures) × 14 (days) factorial, with days considered repeated measurements. In the six vole-only enclosures, baits were removed from the brushed-dirt plots and replaced with four 0% or 2% Zn_3P_2 baits (one per 232.6-cm^2 quadrant; 32 per enclosure); these 'placed' baits were then monitored daily for removal, while the surfaces of all plots were monitored daily for the presence:absence of animal/bird sign. Key results were: (a) 3.51 (±2.66) and 3.39 (±3.52) mean (±SD) baits were found on plots after pre-bait and test-bait broadcasts, respectively—less than the predicted 4.52 particles per 930-cm^2 plot; (b) baits 'placed' on plots in placebo-baited enclosures were removed earlier than those in Zn_3P_2-baited enclosures—data in agreement with observed vole mortality; and (c) species × bait interactions occurred for both the vole- and pheasant-sign counts, but not quail-sign counts—data also indirectly confirming Zn_3P_2-induced mortality effects on voles and pheasants. This technique has utility for a variety of wildlife biology and chemical registration studies; although limited to arid conditions, the technique affords useful indices of broadcast calibration, bait pick-up, as well as target and non-target species mortality. Published in 2002 for SCI by John Wiley & Sons, Ltd

Keywords: index; sign; wildlife abundance; voles; avian hazards; rodenticide; zinc phosphide; bait broadcast; equipment calibration

1 INTRODUCTION

Product Performance Guidelines (GDLN) 96-12 and 71-5 provide recommended methods and endpoints for efficacy and wildlife-hazards studies needed to register agrochemicals in the USA.[1,2] [Note. These GDLN are under revision but, at the time of submission of this article, new, harmonized Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines (ie OPPTS 810 Series for all but invertebrate products and OPPTS 850.2500—Ecological Effects Test Guidelines) remain as public drafts (see http://www.epa.gov).[3]] However, study directors have latitude to devise and to use novel data collection procedures that will aid evaluation of related effects. For example, the methods outlined in GDLN 96-12(e) for field efficacy studies and development of a Section 3 (federal) rodenticide label state that registrants/researchers should: (i) submit five or more studies for each formulation, site, method of application, major region and species associated with use of the rodenticide, (ii) perform pre- and post-treatment population censuses of rodents [ie either direct (capture, mark, release, recapture) or indirect (opened or closed mounds/burrows)], (iii) use separate control ('placebo') sites, (iv) include trials involving different rates, frequencies and modes of application for baits, and (v) conduct studies with environmental factors (eg humidity, rainfall, temperature) similar to those expected for future use conditions.[1]

The OPPTS 850.2500 proposes two approaches to wildlife-hazards tests—screening and definitive—where definitive tests are expected to quantify 'the

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baits were counted daily, but removed for plot-maintenance activities (replaced afterwards) throughout the 14-day test-bait period. These data were viewed as indexing the temporal pattern of bait pickup, and indirectly vole abundance within each vole-only enclosure.

In the vole–pheasant and vole–quail enclosures, baits were counted and removed. No baits were placed onto plots within pheasant and quail enclosures. This avoided confounding of potential non-target-hazards effects attributed to bare-ground exposure of baits.[4]

During the 14-day test-bait period, plots were observed for presence: absence (1:0) of rodent and avian sign each morning (~0800–0900 h), then prepared for the next reading (re-brushed). Species signs on plots were not mutually exclusive; it was possible to detect both vole and pheasant or quail signs on a single plot, but discrimination between pheasant and quail was difficult. Footprints and tail drags of voles were distinctive, but discrimination of vole sign from other potential rodents was not attempted (ie deer mouse, house mouse, etc). The trap out of enclosures prior to the study assured that most rodent signs were voles. Pheasant and quail activity consisted mainly of footprints, feathers, soil scratches and fecal droppings.

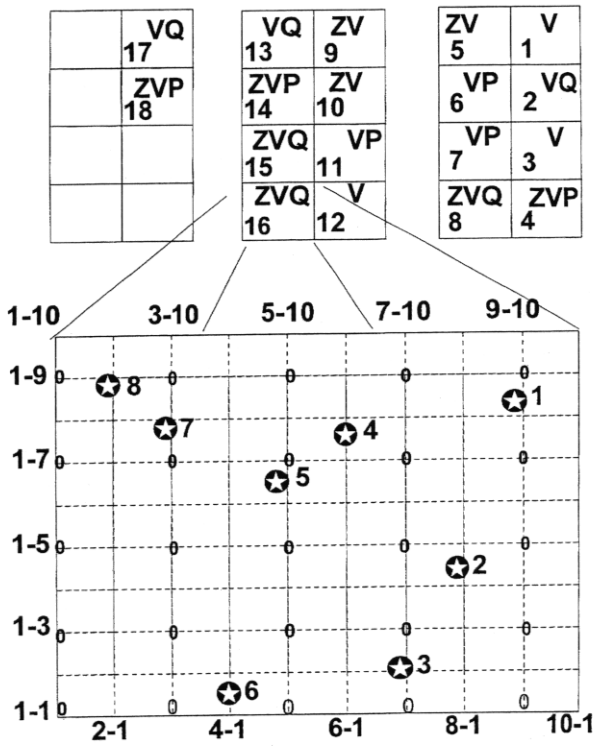

Figure 1. Schematic diagram (not to scale) of the design showing the 18 enclosures (top; six unused enclosures are unnumbered); respective bait (Z=zinc phosphide; placebo is not listed) and species (V=vole; P=pheasant; Q=quail) designations are contained within the enclosure boxes. An exploded view of Enclosure 16, with a trap grid and random locations of eight × 930-cm^2 brushed-dirt plots is also below the schematic (ie Sherman trap locations are at the intersection of each row × column or wall of the dotted lines; pit-fall trap locations are shown as 0; dirt-plot locations=stars within circles).

Size of footprints and coloration of feathers for pheasants and quail were distinctive, soil scratches and feces were not distinctive.

2.3.1 *Initial broadcast equipment calibrations*

Each CPA performed two or three calibration trials prior to both the pre-bait and test-bait broadcasts in adjacent, unused enclosures (see Fig 1).

Calibration trials involved the use of 2072-g and 1906-g quantities of placebo bait, respectively, the amounts being based on the rate of 11.2 kg ha^{-1} and the area to be baited per enclosure. Pre-bait trials assumed a 43 m × 43 m crop of alfalfa (0.1849 ha) with a ~1-m-wide 'no plant' edge; test-bait trials assumed 41 m × 41 m of alfalfa (0.1681 ha) with a 3-m-wide 'no bait' edge (ie baited areas were decreased for test-bait broadcasts to avoid dispensing baits onto bare ground, thereby lowering risks of gallinaceous birds ingesting baits).

Baits were weighed into plastic bags, then emptied into the Spyker® hopper prior to broadcast. The CPAs adjusted the 'spread-width' (~2.5 m) and 'spread-rate adjustment' screws (moderate rate) on the spreaders to settings expected to yield roughly a rate of 11.2 kg ha^{-1}. Next, each CPA began walking and cranking the Spyker® 'delivery crank' within an unused enclosure, making a series of continuous back-and-forth passes in adjacent trap-row paths while broadcasting baits onto each ~5-m-wide alfalfa strip between trap rows. The CPAs were instructed to adjust their 'pace' and 'crank' rates so as to spread half of the hopper contents over half of the alfalfa within the enclosure. Upon completion of eight swaths (ie first four trap-rows), the remaining bait was weighed. Adjustments to the spread-width and spread-rate screws on the spreaders were performed between successive calibration trials. Calibration accuracy was based on the quantity of bait dispensed (ie 50% of the pre-weighed baits should have been broadcast).

Data for the pre-bait trials showed that CPA 1 broadcast 1123 g (46%), 729 g (35%) and 880 g (42%) of pre-weighed baits (mean 41%) during three trials; CPA 2 broadcast 1631 g (79%) and 1042 g (50%) of baits (mean 64%) during two trials.

Data for the test-bait trials showed that CPA 1 dispensed 914 g (48%), 1012 g (53%) and 1062 g (56%) of baits (mean 52%) during the ~0.1-ha practice broadcasts, whereas CPA 2 broadcast 933 g (49%), 713 g (38%) and 1041 g (55%) of baits (mean 48%) during these trials.

2.3.2 *Expected baits on plots*

A mean (±SD) weight of 23 (±9) mg has been reported for 100 randomly selected steam-rolled-oat-groat (SRO) particles prepared similarly to the current placebo bait.[13] Assuming a homogeneous broadcast pattern, arithmetic computations for the 2025 m^2 enclosures based on this mean particle weight would predict the homogeneous dispensing of 4.52 SRO per

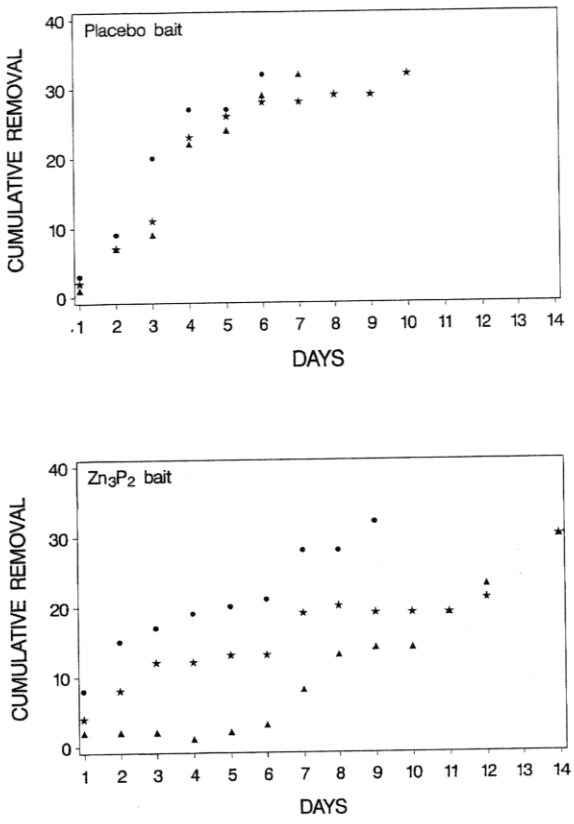

Figure 3. Plots of cumulative bait removals across days in (top) placebo- and (bottom) Zn_3P_2-baited enclosures. [Data are plotted until the criterion of 32 baits were removed; note the steeper slope (rapid removal) for the placebo *versus* the gradual slope (slower removal) for the Zn_3P_2 baits.].

only, quail–vole and pheasant–vole enclosures between placebo- and Zn_3P_2-bait applications (Fig 4). Interpretation is straightforward; this is essentially rodenticide-induced mortality. Evidence of vole abundance or activity was reduced in Zn_3P_2 and gallinaceous-bird enclosures, with the rodenticide causing a major portion of the reduced sign, but scratching and dusting by the pheasants and quail also obscuring some vole sign.

The species main effect showed that mean (±SD) plots with vole sign in vole-only, vole–pheasant and vole–quail enclosures were 4.04 (±2.52), 1.51 (±1.50) and 2.42 (±1.99), respectively. The bait main effect was due to a twofold difference of mean (±SD) vole counts in placebo- *versus* Zn_3P_2-baited enclosures [ie 3.90 (±2.28) and 1.42 (±1.50), respectively]. Regarding the day effect, Tukey tests confirmed the gradual decline in observed vole sign during the test-bait period. Three sets of Tukey mean differences were noted: day 1 vole counts (3.94) were greater than all other days; days 2 (3.44), 4 (3.44) and 9 (3.22) were greater than remaining day means; and days 3 (3.06), 5 (3.00) and 11 (2.83) were greater than remaining means. Thus, despite several slight departures from purely consecutive daily decreases in mean vole counts, the pattern generally concurs with rodenticide efficacy (ie vole mortality in half of the enclosures across time led to decreased mean vole-sign counts later in the bait-exposure period).

3.4 Pheasant and quail activity effects

The ANOVA for avian sign on plots yielded a species × bait ($F = 4.36$; 2, 12 *df*; $P < 0.0378$) interaction, as well as species ($F = 26.22$; 2, 12 *df*; $P < 0.0001$) and day ($F = 2.00$; 12, 143 *df*; $P < 0.0279$) main effects; no other effects, including the main effect for bait ($F = 1.05$; 1, 12 *df*; $P > 0.326$), were significant.

The species × bait interaction was attributed to the crossover in mean avian-sign counts for plots within the vole–pheasant and vole–quail enclosures between placebo- and Zn_3P_2-baited conditions (Fig 5). Mean (±SD) counts for the vole–pheasant placebo and Zn_3P_2 enclosures were 5.82 (±2.15) and 3.12 (±2.88), respectively; whereas the counts for vole–quail enclosures averaged 3.23 (±2.20) and 4.43 (±1.62) between placebo and Zn_3P_2 conditions. As expected, vole-only enclosures were essentially free of pheasants and quail [ie mean (±SD) plots with bird sign for placebo- and Zn_3P_2-baited enclosures were 0.08 (±0.35) and 0.02 (±0.16), respectively—a few escapes of pheasants to these enclosures occurred].[11]

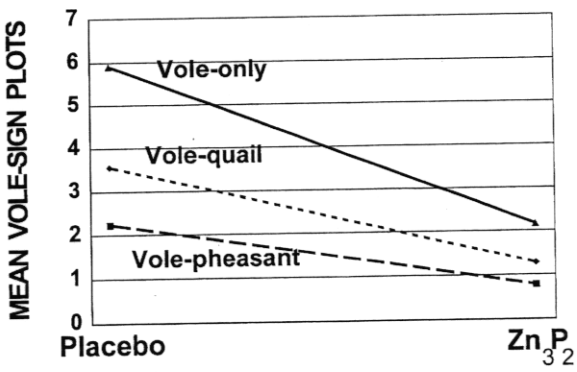

Figure 4. Graph of the species × bait interaction effect characterizing the vole-sign activity on plots within pheasant–vole, quail–vole and vole-only enclosures.

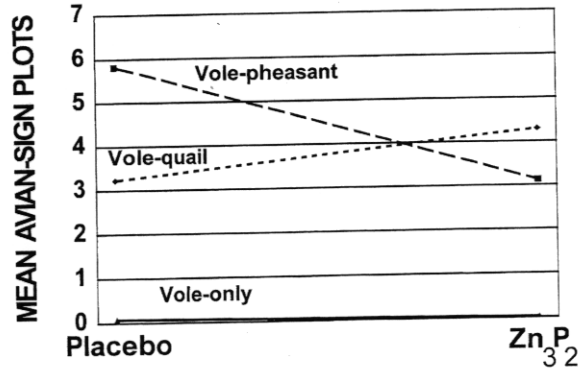

Figure 5. Graph of the species × bait interaction characterizing the avian-sign activity on plots within pheasant–vole, quail–vole and vole-only enclosures.

Use of trade names does not constitute endorsement by the Federal Government.

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